RESEARCH PAPER

Magnesium oxide nanoparticle effects on the central nervous system biochemical parameters and memory deficit induced by a sleep-deprivation

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ABSTRACT

Objective(s): Magnesium plays an important role in the correct functioning of the nervous system and it is hardly able to cross the blood-brain barrier. Magnesium oxide nanoparticles (MgO NPs) can affect memory in animal models. The current study aimed to evaluate the mechanism of action and efficacy of MgO NP in comparison to conventional magnesium oxide (C MgO) in learning and memory at the sleep-deprived model of rats.

Materials and Methods: Adult male Wistar rats (200±20 gr) were divided into control, MgO NP and C MgO (1, 5, and 10 mg/kg) groups. Short-term and long-term memories were evaluated by the passive avoidance test. The Columns-in-water method was used to induce sleep deprivation (SD) for 72 h in all groups. Oxidative stress markers including glutathione, glutathione peroxidase, Malone di-aldehyde, total antioxidant capacity, catalase activity, superoxide dismutase, and brain derived neurotropic factor (BDNF) were assessed in the hippocampus of all animals. Also, brain and hippocampus magnesium levels were evaluated in all groups.

Results: MgO NP (5 and 10 mg/kg) significantly improved short and long-term memory impairment-induced by SD ($P<0.05$). Hippocampus magnesium levels increased in all groups treated by MgO NP. There were no significant changes in the hippocampal oxidant and anti-oxidant factors level and BDNF in MgO NP and C MgO treated groups.

Conclusion: Probably MgO NP could entrance the brain and the gathering of magnesium ions in the hippocampus enhanced memory. So that memory improvement can be related to the increasing magnesium level in the hippocampus that this needs more research.

Keywords: *Memory, Nanoparticle, Rat, Sleep deprivation*

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INTRODUCTION

Sleep plays an important role in the normal function of the central nervous system and there is a relationship between sleep and memory; also, sleep deprivation (SD) impairs memory retention [1-5]. Our previous study indicated that 72 hr pre-training SD impairs passive avoidance memory retention in the male rats [6].

Magnesium is one of those supplements that is very well known for its benefits throughout the natural health community and plays an important role in the correct functioning of the nervous system. There is a positive correlation between brain magnesium level and enhancement of learning and memory and magnesium supplementation can enhance sleep indices too [7, 8].

Magnesium deficiency can cause insomnia and there is a close relationship between the amount of magnesium and the quality of sleep [9]. Metal oxide nanoparticles such as MgO NPs, as a source of magnesium, are involved in drug delivery and medicine, but there is evidence that metal oxide nanoparticles induce oxidative stress [10, 11].

It has been indicated that the administration of MgO NP after training prevents morphine-

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induced amnesia [11]. Post-training injection of MgO NP improved the long-term memory in mice and this nanoparticle improved atropine-induced memory impairment [12]. MgO NP has improved memory impairment induced by streptozotocin in Alzheimer-like model of rats [8].

Due to the above results, it seems that MgO NPs could improve memory impairment, but the efficacy of MgO NP on memory impairment induced by sleep deprivation is not clear yet.

On the other hand, BDNF is a neurotropic factor that enhances the growth and differentiation of neurons and synapses, and elevation of brain magnesium increased BDNF expression [13]. Some studies have indicated that BDNF plays a prevention role in dementia and inflammation and serum BDNF levels are lower in Alzheimer's disease [14, 15]. Magnesium can increase the hippocampal level of BDNF in rats and sleep deprivation can reduce BDNF level too [16, 17].

Even though there are various forms of memory impairment, but little information has been found about memory deficits due to insomnia in the presence of magnesium in conventional and nano forms, and MgO NP function in the memory improvement is unknown yet. Since insomnia can cause oxidative stress [18], this study was designed to investigate and compare the effect of MgO NPs and C MgO on memory impairment induced by sleep deprivation and oxidative and antioxidant markers, magnesium and BDNF levels in the hippocampus of male rats.

MATERIALS AND METHODS

Animals and components

In this experimental study, male Wistar rats (weighing 200±20 g) were housed under a 12-12 *h* light-dark schedule and standard conditions of temperature (23 ± 1 °C), with free access to the food and water.

Rats were randomly divided into groups of control (saline 0.9%), MgO NP (1, 5 and 10 mg/kg) and C MgO (1, 5 and 10 mg/kg) [8]. The number of animals in each group was 6-7 (N=6-7). The experimental protocol was designed in Fig. 1. MgO NP (US Nano Co, USA, particle size <20 nm, Stock: US3290) and MgO (Merck Co, Germany, particle size >100 nm) were dispersed in 0.9% saline and administered intraperitoneally (i.p.) in a volume of 3 mL/kg of body weight. The control group just received 0.9% saline. Fig. 2 shows the intensity of the reflection in the X-ray pattern is related to MgO NP.

All procedures were performed in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of the Shahid Chamran University of Ahvaz, Iran (Code: EE/96.24.3.88375/scu.ac.ir).

Sleep deprivation

Multiple platforms method (columns-in-water) used for the model of sleep deprivation as previously described [6]. Rats were placed in an aquarium filled with water at room temperature and sleep deprivation was accomplished for 72 hr

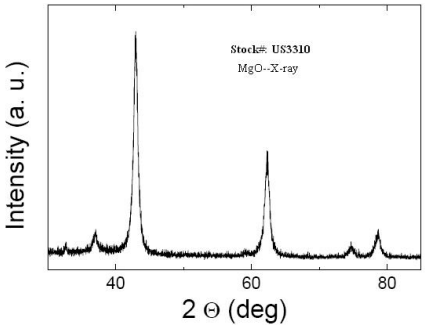

Fig. 2. X-ray pattern of MgO NP. Diffraction angle is between 35° and 85°

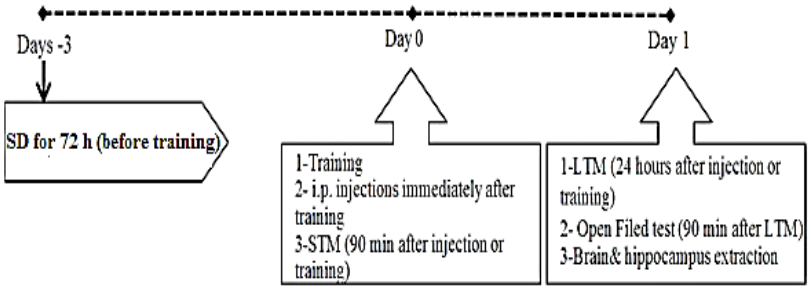

Fig.1.Experimental protocol. Abbreviation: SD= sleep deprivation, i.p= intraperitoneal, STM=Short-term memory, LTM= Long-term memory

before the training session [6].

Passive avoidance protocol

The step-through passive avoidance (PA) was performed as described previously [6]. During PA habituation, rats were placed in the white compartment facing away from the dark compartment and allowed to explore for 10 sec, then the door was raised and the rat was allowed to explore freely. When the rat entered the dark compartment with all four paws, the guillotine door was closed, and the latency to enter was recorded. During PA training (30 min after habituation), rats were placed in the white compartment facing away from the dark compartment and allowed to explore for 10 sec. Thereafter, the sliding door was opened and once the rat had entered the black compartment, the sliding door was closed and a weak electrical foot-shock (0.5 mA, 5 sec duration) was delivered. After the foot shock, the rat is removed from its home cage. After 90 min (short-term memory (STM) test) and 24 hr (long-term memory (LTM) test), the animal was again gently placed in the white compartment facing away from the dark compartment, after 10 sec, the door was lifted, and the latency to enter the black compartment with all four paws (retention latency) and the period of staying in the black compartment were measured with a cut off time for testing after 300 s. After step-through passive avoidance test, an open field apparatus was used to assess the locomotor activity of animals. Each animal was placed in the center of the apparatus and was left free to explore the arena for 5 min adaptation phase. After adaptation, the crossing number in 3 min was counted.

Hippocampus and brain biochemical factors

After the tests, all animals were sacrificed according to the guidelines using chloroform, the brain was removed and the hippocampus was dissected. The tissues were homogenized in phosphate buffer (5-time tissue weight) and the homogenate were centrifuged to remove the insoluble materials (14,000×g for 15 min at 4 °C).

Biochemical tests

Total protein assay: The total protein concentration of the hippocampus was estimated using the Bradford procedure [19].

BDNF assay: Hippocampus BDNF levels were measured using enzyme-linked immunosorbent assay using Rat BDNF ELISA (Enzyme-Linked Immunosorbent Assay) Kit (CK-E30666 produced by East Biopharma).

Total antioxidant activity (TAC) assay: TAC was measured by the ferric reducing ability of plasma (FRAP) according to the method is described by Benzie and Strain, 1996 [20].

SOD assay: Superoxide dismutase (SOD) activity in the homogenate hippocampus was assayed according to the method is described by Kono, 1978 [21].

GPx assay: Glutathione peroxidase (GPx) activity was measured in homogenates by the Ransel kit (Randox, UK).

Catalase assay: Catalase activity was assayed in homogenates according to the method is described by Koroluk et al., 1998 [22].

GSH assay: The Glutathione (GSH) levels were measured according to the method is described by Ellman, 1959 [23].

Lipid peroxidation assay: Malondialdehyde (MDA) results from the degradation of polyunsaturated fatty acids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation was determined [24].

Magnesium assay: The brain and hippocampus magnesium levels were determined using a magnesium kit (Pars Azmoon, Iran).

A BioTek spectrophotometer (XS2 model, America) and Gen 5 software were used to measure and analyze the wavelength absorption.

Statistical analysis

One-way analyses of variance (ANOVA), Post-hoc of Tukey, student's T-test and, Pearson's correlations were utilized to analyze the collected data by Instat 3 software. Statistical significance was set at the $P<0.05$ level. Results are presented by the Mean± standard error of the mean (S.E.M).

RESULTS

Effects of C MgO and MgO NPs on short and long term memory in 72 hr pre-training SD rats

Fig. 3 indicates post-training injection of MgO NPs improved short-term (A) and long-term (B) memory by increasing the latency to enter the dark compartment, in a dose depending manner, and this effect was significant at the dose of 10 mg/kg in short ($F(3, 20) =4.898$; $P<0.0103$) and long term ($F(3, 20) =5.123$; $P<0.0086$) memory. C MgO improved relatively the short-term ($F(3, 20) =2.793$; $P< 0.0668$) and long-term ($F(3, 20) =2.793$;

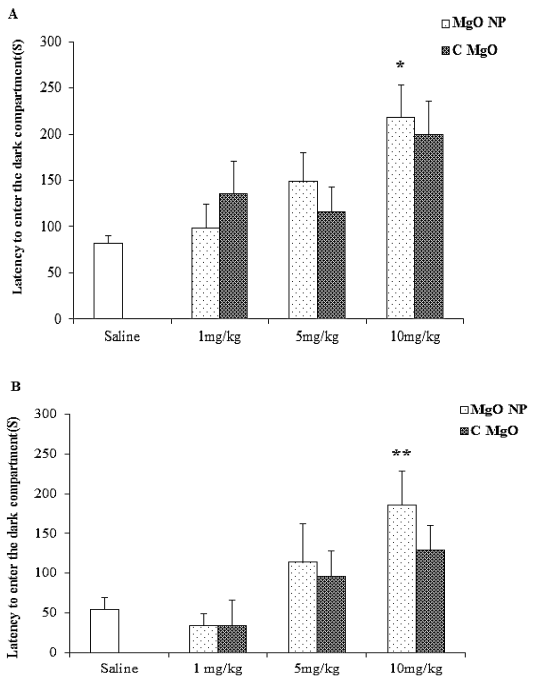

Fig. 3. Effects of C MgO and MgO NP on latency to enter the dark compartment in short-term memory (A) and long-term memory (B). *P<0.05 and **P<0.01 are compared to the saline group

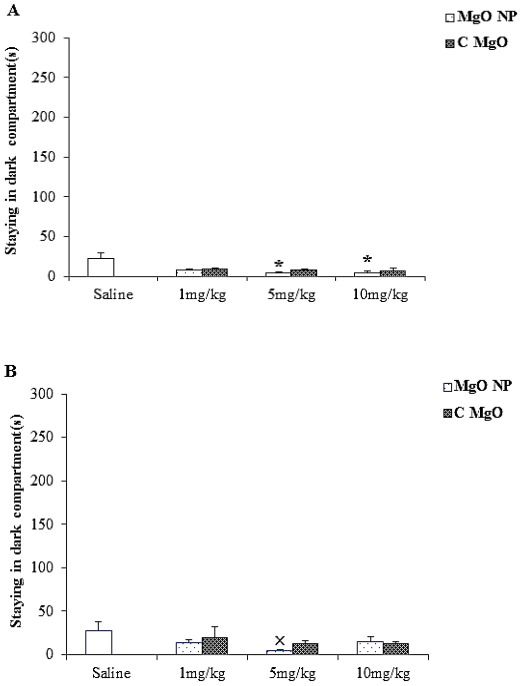

Fig. 4. Effects of C MgO and MgO NP on staying in a dark compartment in short-term memory (A) and long-term memory (B). *P<0.05 is compared to the saline group. × P<0.05 is compared to the same dose of C MgO

P<0.0668) memory with a dose dependent manner, but was not statistically significant difference between the latency to enter the dark compartment in short and long term memory for the same dose of components including Saline: t(10)=1.599; P<0.141, MgO NPs 1: t (10) = 2.147; P<0.0574, MgO NPs 5: t(10)= 0.5942; P<0.5656, MgO NPs 10: t(10) = 0.3733; P< 0.7167, C MgO 1: t (10)= 2.094; P<0.0627, C MgO 5: t(10)= 0.449; P< 0.663 and C MgO 10: t(10) = 1.453; P<0.1808. Fig. 4 shows MgO NP improved short-term (A(F(3, 20) =4.25; P<0.0178)) and long-term (B(F(3, 20) =2.244; P<0.1145)) memory by reducing staying in dark compartment and this effect was significant for MgO NP in short-term memory and had no effect on long-term memory.

In t-test for same doses of MgO NP and C MgO 1 mg/kg (t (10) =0.2559; P<0.8032), 5mg/kg (t(10) =2.427; P<0.0356) and 10 mg/kg (t(10) =0.6532; P<0.5284), only MgO NP 5 mg/kg reduced staying in black compartment in comparison with C MgO.

Effects of C MgO and MgO NP on motor activity

Fig. 5 shows that different doses of C MgO and MgO NPs had no effect on motor activity in the open field test, only C MgO 10 mg/kg reduced motor activity in comparison with saline (t(10=2.821; P<0.018) and MgO NPs 10 mg/kg (t (10) =3.924; P< 0.0028), (ANOVA for all doses of MgO NP and the saline group was F (3, 20) =0.8473; P<0.4842, ANOVA for all doses of C MgO and saline was F (3, 20) =2.097; P<0.1327).

Effects of C MgO and MgO NP on hippocampus biochemical factors

Hippocampus biochemical data are shown in Table 1.

Catalase Activity: There were not significant

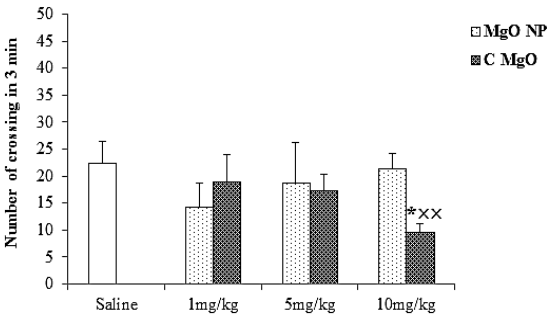

Fig. 5. Effects of C MgO and MgO NPs on motor activity. *P<0.05 is compared to the saline group. ×× P<0.01 is compared to the same dose of MgO NP

Table 1. Effects C MgO and MgO NP on hippocampus biochemical factors

Factors	Catalase	TAC	GSH	GPX	SOD	MDA	BDNF
	(umol/mg pr/min)	(umol/mg pr)	(umol/mg/pr)	(umol/mg pr)	(umol/mg pr/min)	(umol/mg pr)	(ng/mg pr)
Groups							
Saline	0.558±0.114	0.838±0.264	0.173±0.0321	1.227±0.110	5.791±0.4287	0.528±0.040	304.094±18.498
MgO NP1 mg/kg	0.358±0.067$^{\times}$	0.477±0.038	0.135±0.010	1.166±0.092	5.181±0.552	0.670±0.039$^{\times}$	328.472±18.221
MgO NP5 mg/kg	0.464±0.095	0.637±0.085	0.165±0.012	1.378±0.122$^{\times}$	6.075±0.447	0.642±0.038	303.913±17.511
MgO NP10 mg/kg	0.774±0.181	0.417±0.081	0.130±0.006	1.223±0.047	5.530±0.602	0.440±0.021$^{\times\times}$	355.816±24.799
C MgO1 mg/kg	0.792±0.159	0.565±0.218	0.107±0.007	1.105±0.071	6.028±0.534	0.527±0.017	314.145±9.352
C MgO5 mg/kg	0.577±0.120	0.486±0.125	0.150±0.012	1.00±0.102	6.875±0.790	0.592±0.043	302.110±9.134
C MgO10 mg/kg	0.412±0.062	0.486±0.049	0.134±0.024	1.175±0.117	5.233±0.549	0.587±0.031	306.474±18.805

× P<0.05 and ××P<0.01 are in comparison with same dose of C MgO
TAC=Total antioxidant activity, GSH= Glutathione, GPx= Glutathione peroxidase, SOD= Superoxide dismutase, MDA= Malondialdehyde, BDNF= Brain derived neurotropic factor

differences between the catalase enzymes activity in control and different doses of MgO NP (F(3, 20)= 2.099; P<0.1324) and C MgO (F(3, 20)= 1.724; P<0.1943) groups. C MgO 1 mg/kg significantly increased catalase enzyme activity compared to MgO NP 1 mg/kg (t (10) =2.503; P<0.0313). But there were no significant differences between C MgO and MgO NP at the doses of 5 and 10 mg/kg (t(10) =0.7313; P<0.4814, t(10) =1.89; P<0.088, respectively).

Total Antioxidant Capacity (TAC): There were not significant differences between the TAC levels in control and different doses of MgO NP (F(3, 20) =2.286; P<0.1098) and different doses of C MgO (F(3, 20) =0.9935; P<0.4161) groups.

T-tests between same doses of MgO NP and C MgO including: 1 mg/kg (t(10)= 0.397; P<0.6997), 5 mg/kg (t(10) =0.9966; P< 0.3425), 10 mg/kg (t(10) =0.7228; P<0.4864), were not significant.

Glutathione (GSH) Levels: there were not significant change in the hippocampal GSH levels among various groups including different doses of MgO NPs (F(3, 20) =1.34; P<0.2896) and different doses of C MgO (F(3, 20) =1.646; P<0.2106). T-test for same doses of MgO NP and C MgO including: 1 mg/kg: t(10) =2.123; P<0.0597, 5mg/kg: t(10) =0.8403; P<0.4204, 10 mg/kg: t(10) =0.1561; P<0.8791, were no significant.

Glutathione peroxidase activity: There were no significant differences between all doses of MgO NPs (F(3, 20) =0.862; P<0.4769) and C MgO (F(3, 20) =0.8848; P<0.4658) on Glutathione peroxidase activity.

Comparison the effect of same dose of components with t-test revealed only glutathione peroxidase activity in MgO NP 5 mg/kg was higher than C MgO 5mg/kg (t(10) =2.335; P<0.0417). T-test between MgO NP and C MgO 1 mg/kg (t(10) =0.5233; P<0.6122) and 10 mg/kg (t(10) =0.3802; P<0.7117) were no significant respectively.

Superoxide dismutase activity: One-way ANOVA on SOD activity revealed no significant effects of pre-training SD in the presence of different doses of MgO NPs (F(3, 20) =0.7902; P<0.5135) and different doses of C MgO (F(3, 20) =1.365; P<0.2821). T- test for the same doses of MgO NP and C MgO were no significant too (1 mg/kg (t(10) =1.348; P<0.2075), 5mg/kg (t(10) =0.8242; P<0.429), 10mg/kg (t(10) =0.4318; P<0. 0.675).

Lipid Peroxidation: The results showed MgO NP (10mg/kg) significantly reduced MDA level compared to other two doses of MgO NP (ANOVA for saline and different doses of MgO NPs: F(3, 20) =7.513; P<0.0015,and ANOVA for saline and different doses of C MgO: F(3, 20) =0.9178; P<0.450).

Comparison the effect of same dose of components with t-test revealed concentration of lipid peroxidation in C MgO 10 mg/kg was higher than MgO NP 10 mg/kg and MgO NP 1 mg/kg was higher than C MgO (1mg/kg (t (10) =2.675; P<0.0233), 5mg/kg (t(10) =0.8413; P<0.4198), 10mg/kg (t(10) =3.647; P<0.0045)).

Brain derived neurotropic factor: Different doses of MgO NP and C MgO had no significant effect on hippocampal BDNF (ANOVA for MgO NPs: F(3, 20) =1.529; P<0.2378, and ANOVA for C MgO: F(3, 20) =0.1282; P<0.9422).

T-test for same doses of MgO NP and C MgO were no significant too (1 mg/kg (t(10) =0.6995;

Table 2. Effects of C MgO and MgO NP on brain and hippocampus magnesium level

Tissue Groups	Brain Magnesium (µg/g of wet tissue)	Hippocampus Magnesium (µg/g of wet tissue)
Saline	63.748±2.994	24.923 ±2.844
MgO NP1 mg/kg	66.672±1.437	27.714 ±0.989
MgO NP5 mg/kg	66.728±4.378	28.050 ±0.7454#
MgO NP10 mg/kg	67.717±3.142	32.782 ±0.801*##
C MgO1 mg/kg	58.008±9.788	25.431 ±1.407
C MgO5 mg/kg	62.159±3.078	24.021 ±1.429
C MgO10 mg/kg	55.104±8.794	26.434 ±1.868

*P<0.05 is compared to the control (saline), #P<0.05 and ##P<0.01 are compared to the same dose of C MgO

P<0.5002, 5 mg/kg (t(10)= 0.09129; P< 0.9291), 10 mg/kg (t(10)=1.586; P<0.1438)).

Effects of C MgO and MgO NP on brain magnesium level

Table 2 shows that different doses of C MgO and MgO NP had no significant effect on brain magnesium in comparison to the saline group (ANOVA for MgO NP: F(3, 16) =0.2932; P<0.8297, and ANOVA for C MgO: F(3, 16) =0.323; P<0.8087).

T-test for same doses of MgO NP and C MgO were: 1 mg/kg: t(10) =0.8757; P<0.4067, 5 mg/kg: t(10) =0.8536; P<0.4181, 10mg/kg: (t(10) =1.351; P<0.2138).

Effects of C MgO and MgO NP on hippocampus magnesium level

Table 2 shows that MgO NP (10mg/kg) increased hippocampus magnesium significantly (ANOVA for MgO NP: F(3, 16) =4.244; P<0.0179, ANOVA for C MgO: F(3, 16) =0.2937; P<0.8295). Comparison the effect of same dose of components with t-test revealed magnesium concentration in MgO NPs 5 mg/kg (t(10) =2.5; P<0.0314) and 10 mg/kg(t(10) =3.636; P<0.0046) were higher than same doses of C MgO and was no difference in dose of 1 mg/kg (t(10) =1.611; P<0.1383).

The result showed the brain magnesium concentration was significantly higher than the hippocampus in all groups. T-tests were significant in saline: (t(9) = 9.364; P<0.0001), MgO NP 1:(t(9)= 24.516; P<0.0001), MgO NP 5: (t(9)= 11.723; P<0.0001), MgO NP 10: (t (9)= 10.668; P<0.0001), C MgO 1: (t(9)= 3.649; P<0.0053), C MgO 5: (t(9) =11.932; P<0.0001), C MgO10: (t(9)= 3.532; P<0.0064).

Pearson's correlations between the memory (latency to enter the dark compartment) and magnesium level in the brain and hippocampus

Table 3 shows, there is a positive correlation between the magnesium concentration in the brain and hippocampus and short-term (90 min after training) and long-term (24 hours after

Table 3. Pearson's correlations between STM and LTM (in latency to enter the dark compartment parameter) with brain and hippocampus magnesium

Groups		Saline	MgO NP1	MgO NP5	MgO NP10	C MgO1	C MgO5	C MgO10
Factors			mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
STM & Brain	R	0.681	0.982	0.683	0915	0.579	0.637	0.725
	P	0.205	0.003**	0.203	0.03*	0.307	0.248	0.166
STM & Hippocampus	R	0.897	0.83	0.741	0.960	0.973	0.695	0.843
	P	0.039*	0.082	0.152	0.009**	0.005**	0.192	0.073
LTM & Brain	R	0.881	0.949	0.558	0.854	0.926	0.725	0.844
	P	0.049*	0.014	0.329	0.066	0.024*	0.166	0.072
LTM & Hippocampus	R	0.319	0.770	0.821	0.893	0.928	0.731	0.953
	P	0.601	0.127	0.088	0.041*	0.028*	0.160	0.012

STM= Short Term Memory; LTM=Long Term Memory

training) memory and these correlations are significant in some groups.

Pearson's correlations between the memory (latency to enter the dark compartment) and hippocampus biochemical factors

Table 4 shows, there is a positive correlation between the hippocampal concentration of BDNF and short-term (90 min after training), and long-term (24 hr after training) and these correlations are significant in some groups. Table 4 also shows there is a negative correlation between the hippocampal concentration of oxidant and antioxidant agents and short-term (90 min after training) and long-term (24 hr after training), and these correlations are significant in some groups.

Table 4. Pearson's correlations between STM and LTM (in latency to enter the dark compartment) with hippocampus biochemical factors

Groups / Factors		Saline	MgO NP1 mg/kg	MgO NP5 mg/kg	MgO NP10 mg/kg	C MgO1 mg/kg	C MgO5 mg/kg	C MgO10 mg/kg
	R	0.480	0.766	0.854	0.737	0.877	0.381	0.846
BDNF & STM	P	0.336	0.131	0.065	0.094	0.051	0.456	0.034*
BDNF & LTM	R	0.556	0.699	0.803	0.766	0.422	0.78	0.782
	P	0.251	0.189	0.102	0.075	0.479	0.067	0.066
Catalase & STM	R	-0.924	-0.046	-0.879	0.112	-0.024	-0.154	-0.892
	P	0.008**	0.942	0.021*	0.833	0.963	0.772	0.017*
Catalase & LTM	R	-0.689	-0.786	-0.914	-0.119	-0.080	-0.388	-0.756
	P	0.13	0.064	0.011*	0.822	0.88	0.448	0.082
TAC & STM	R	-0.551	-0.737	-0.902	-0.926	-0.792	-0.176	-0.691
	P	0.336	0.095	0.014*	0.008	0.061	0.738	0.129
TAC &LTM	R	-0.046	-0.91	-0.831	-0.817	-0.504	0.087	-0.626
	P	0.942	0.012*	0.04*	0.047*	0.308	00869	0.184
GSH & STM	R	-0.076	-0.67	-0.734	-0.606	-0.715	-0.893	-0.819
	P	0.886	0.145	0.097	0.202	0.11	0.017*	0.046*
GSH & LTM	R	-0.358	-0.843	-0.686	-0.432	-0.56	-0.646	-0.705
	P	0.486	0.035*	0.133	0.392	0.248	0.166	0.118
GPx & STM	R	-0.767	0.031	-0.617	-0.812	0.029	-0.763	-0.693
	P	0.075	0.953	0.192	0.05*	0.956	0.077	0.127
GPx & LTM	R	-0.722	-0.515	-0.566	0.787	-0.189	-0.52	-0.472
	P	0.105	0.296	0.241	0.063	0.72	0.291	0.344
SOD & STM	R	-0.847	-0.535	-0.533	-0.911	-0.784	-0.178	-0.206
	P	0.023*	0.274	0.276	0.012*	0.065	0.735	0.695
SOD &LTM	R	-0.771	-0.502	-0.377	-0.882	-0.669	-0.55	-0.496
	P	0.073	0.311	0.462	0.02*	0.146	0.258	0.317
MDA& STM	R	-0.881	-0.549	-0.572	-0.634	-0.909	-0.262	-0.847
	P	0.02*	0.259	0.236	0.176	0.012*	-.617	0.033*
MDA& LTM	R	-0.967	-0.354	-0.535	-0.358	-0.587	-0.013	-0.691
	P	0.002**	0.491	0.274	0.486	0.221	0.981	0.129

BDNF= Brain derived neurotropic factor, TAC= Total antioxidant activity, GSH= Glutathione, GPx= Glutathione peroxidase, SOD= Superoxide dismutase, MDA= Malondialdehyde, STM= Short Term Memory; LTM=Long Term Memory

DISCUSSION

The results of the present study showed that MgO NPs improved memory impairment-induced by 72 hr pre-training SD in a dose-dependent manner, while C MgO had a partially effect on memory. There were positive correlations between the brain and hippocampus magnesium concentration, the hippocampal concentration of BDNF and memory, and a negative correlation between the hippocampal concentration of oxidant and antioxidant agents and memory.

Nanotechnology helps in the treatment of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [25]. It has been shown that, acute and chronic administration of MgO NP significantly improved short and long-term memory in the Alzheimer-like model of rats and prevented the mechanisms of morphine-induced amnesia [8,10]. It has been shown that MgO NPs have the anxiolytic effect; while C MgO did not have any effect [26].The above evidence confirms our current results on better efficacy of MgO NP rather than C MgO in memory improvement.

In this study, highest dose of MgO NP increased hippocampus magnesium level significantly and there was a positive correlation between magnesium level in the hippocampus and memory improvement. Numerous studies have described the effect of magnesium on sleep and memory and Alzheimer's disease seems to be associated with a lower magnesium status [15, 16. 27, 28]. It has been shown that elevation of brain magnesium (with using magnesium-L-threonate, MgT) enhances synaptic plasticity in the hippocampus and memory retention of the extinction of fear memory in rats [15]. In association with the magnesium-enhanced memory mechanism, Kesmati et al., 2021 have shown that MgO NP improved streptozotocin-induced cell lesions in different parts of the hippocampus of male rats in the Alzheimer-like model, as well as, MgO NP significantly improved memory.

On the other hand, there is evidence that nanoparticles induce oxidative stress [10], while magnesium has neural protection roles [29, 30]. The results of our study revealed that sleep deprivation, the use of MgO NP or a combination of both, does not produce intense oxidative stress and didn't cause significant changes in the hippocampal oxidative stress markers and antioxidant enzymes but there is a negative correlation between these markers and memory.

Mathangi et al., 2012 showed that 96 hours of REM sleep deprivation increased lipid peroxidation and reduction in total reduced glutathione levels in the discrete regions of the brain studied [31]. Coenen and Van Luijtelaar, 1985 have shown that the multiple platforms method produces less oxidative stress compared to a single platform technique, due to a reduction of movement restriction in the latter group and induces only mild stress [32]. Besides, voluntary locomotors activity (in multiple platforms) decrease oxidative damage associated with isolation stress and longer durations of locomotors activity were associated with less oxidative stress [33]. Despite the negative correlation between different oxidant and antioxidant factors and memory, in our study, the changes in these factors due to sleep deprivation were not significant, and this can be due to the possibility of moving animals in a multi-platform approach.

Also, our study showed that different doses of C MgO and MgO NPs have no effect on the hippocampal BDNF protein and the improvement effect of MgO NP on memory was not associated with a change in hippocampal BDNF level. Alzoubi et al., 2013 have shown that sleep deprivation and the Western diet (combined effect) reduced the level of hippocampal BDNF, and this reduction was not observed with sleep deprivation alone [18]. Torabi-Nami et al., 2014 revealed that serum BDNF was significantly reduced in TSD (total sleep deprivation) and CPSR (chronic partial sleep restriction) in the rat [34]. Pochwat et al., 2015 showed that administration once daily for 14 days magnesium significantly increased the levels of BDNF in the prefrontal cortex and the hippocampus in olfactory bulbectomy rats [17]. It has been shown that the application of antenatal $MgSO_4$ in preterm delivery increased cord blood BDNF levels, which could have a potential role in fetal neuroprotection [35]. Hairston et al., 2004 showed that BDNF was increased by sleep deprivation in the cortex and hippocampus of rats [36]. Contradiction in the above results with our study can be returned to the importance of the site of BDNF measurement.

CONCLUSION

The results of this study revealed that the MgO NP administration leads to more magnesium entrance to the brain and gathering of this ion in the hippocampus and improvement of

memory impairment-induced by SD. Also, the use of this nanoparticle does not produce severe oxidative stress and does not alter the amount of hippocampus BDNF. Therefore consumption of supplements that increase the amount of magnesium in the brain can reduce the side effects of sleep deprivation on memory, and increasing the amount of brain magnesium by MgO nanoparticles can be a new and useful strategy for enhancing cognitive abilities.

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